

MRID No. 415780-02

## DATA EVALUATION RECORD

1. **CHEMICAL:** Didecyl Dimethyl Ammonium Chloride.  
Shaughnessey Number: 69149.
2. **TEST MATERIAL:** 1) Nonlabeled Didecyldimethylammoniumchloride (DDAC); BARDAC 22; Lot No. 7807-E; 41.2% active ingredient; a light yellow liquid. 2) Radiolabeled  $^{14}\text{C}$ -Didecyldimethylammoniumchloride ( $^{14}\text{C}$ -DDAC); BARDAC 22; Lot No. 7499-E; >99% pure; specific activity of 9.40 mCi/mmol; a clear liquid.
3. **STUDY TYPE:** Freshwater Invertebrate Static Acute Toxicity Test. Species Tested: *Daphnia magna*.
4. **CITATION:** LeLievre, M.K. 1990. Evaluation of Didecyldimethylammoniumchloride (DDAC) in a Static Acute Toxicity Test with Daphnids, *Daphnia magna*. Prepared by Springborn Laboratories, Inc., Wareham, Massachusetts. SLI Report #89-10-3112. Study #11696.0887.6102.110. Submitted by Lonza, Inc., Fair Lawn, New Jersey. EPA MRID No. 415780-02.
5. **REVIEWED BY:**  
  
Kimberly Rhodes, M.S.  
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Signature: *Kimberly Rhodes*  
Date: *May 4, 1992*
6. **APPROVED BY:**  
  
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KBN Engineering and  
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Date: *5/4/92*  
  
Henry T. Craven, M.S.  
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Signature: *John Niles*  
Date: *9/24/92*
7. **CONCLUSIONS:** This study appears to be scientifically sound and meets the requirements for a static acute toxicity test using freshwater invertebrates. Based on mean measured concentrations, the 48-hour  $\text{EC}_{50}$  value of DDAC for *Daphnia magna* was  $94 \mu\text{g a.i./l}$ , which classifies DDAC as very highly toxic to *Daphnia magna*. The NOEC was  $74 \mu\text{g a.i./l}$  mean measured concentration.
8. **RECOMMENDATIONS:** N/A.

9. BACKGROUND:10. DISCUSSION OF INDIVIDUAL TESTS: N/A.11. MATERIALS AND METHODS:

- A. Test Animals: *Daphnia magna* ( $\leq 24$  hours old) were obtained from in-house cultures. The cultures were maintained in a temperature-controlled area ( $20 \pm 2^\circ\text{C}$ ) on a 16-hour daylight photoperiod at a light intensity of 80-120 footcandles at the solution surface. The culture water was from the same source as the dilution water and had similar physical characteristics.

Daphnids were fed a combination of a trout food suspension and green algae (*Ankistrodesmus falcatus*) once daily.

- B. Test System: Each test vessel had a surface area of  $78.5\text{ cm}^2$  and contained 1000 ml of test solution. The solution depth was 12.7 cm. The ambient air temperature in the laboratory was controlled in order to maintain test solution temperatures at  $20 \pm 1^\circ\text{C}$ . The photoperiod was the same as that used in culturing with a light intensity of 60 footcandles at the solution surface.

The dilution water was prepared by fortifying well water according to the ASTM (1980) and filtering it through an Amberlite XAD-7 resin column to remove any organic contaminants. The dilution water had a total hardness of 170 mg/l as  $\text{CaCO}_3$ , a total alkalinity of 120 mg/l as  $\text{CaCO}_3$ , a pH of 8.2, and a specific conductivity of 500  $\mu\text{mhos/cm}$ . Routine analyses were conducted on representative samples of the dilution water and food source for the presence of pesticides and PCB's.

A 1.0 mg a.i./ml stock solution was prepared by diluting 0.0213 g of nonlabeled DDAC with 3.8 ml of a 0.350 mg a.i./l  $^{14}\text{C}$ -radiolabeled DDAC stock solution and bringing the volume to 10 ml with distilled water.

- C. Dosage: Forty-eight-hour acute static test. Based on the results of a preliminary test, five nominal concentrations (13, 22, 37, 60, and 100  $\mu\text{g}$  a.i./l) were tested. A dilution water control was also included in the test.

- D. **Design:** Twenty daphnids were impartially distributed to each concentration (ten daphnids per replicate) within thirty minutes after the test solutions were prepared. Daphnids were not fed during the test.

Observations of immobilization and abnormal effects were recorded at 24 and 48 hours of exposure. The dissolved oxygen concentration, temperature, and pH were measured once daily in one replicate vessel of each concentration and the control. Total hardness, alkalinity, and specific conductivity were measured at test initiation in one replicate vessel of the highest test concentration and control. The temperature was also continuously monitored throughout the study using a min/max thermometer.

Water samples were collected every 24 hours from each test concentration and control. Quality control and test samples were analyzed for  $^{14}\text{C}$ -DDAC by liquid scintillation counting techniques.

- E. **Statistics:** The median effect concentration ( $\text{EC}_{50}$ ) and associated 95% confidence interval for each 24-hour interval were calculated using a computer program that employed multiple methods of analysis (i.e., probit analysis, moving average angle, and binomial probability). The no-observed-effect concentration (NOEC) was defined as the highest concentration tested at and below which there were no toxicant-related physical and behavioral abnormalities.

12. **REPORTED RESULTS:** Mean measured concentrations were 19, 31, 51, 74, and 120  $\mu\text{g a.i./l}$  DDAC and ranged from 120 to 146% of nominal concentrations (Table 2, attached). Analysis of the dilution water and food source showed no detectable amounts of pesticides or PCB's.

The cumulative percent immobilization and observations made during the test are presented in Table 3 (attached). The 48-hour  $\text{EC}_{50}$  was determined to be 94  $\mu\text{g a.i./l}$  with a 95% confidence interval of 74-120  $\mu\text{g a.i./l}$ . The NOEC established for this study was 74  $\mu\text{g a.i./l}$ . "However, immobilization of 5% was observed in the 31  $\mu\text{g a.i./l}$  test concentration and the control at 48 hours of exposure, and one of the exposed organisms in the 74  $\mu\text{g a.i./l}$  treatment level exhibited lethargic behavior."

During the study, pH ranged from 7.8 to 8.5 and the dissolved oxygen concentration ranged from 7.3 to 9.3 mg/l (80% to 102% of saturation). The total hardness and alkalinity of the highest test concentration at test

initiation were 180 and 120 mg/l as  $\text{CaCO}_3$ , respectively, and the specific conductivity was 550  $\mu\text{mhos/cm}$ . Continuous monitoring throughout the exposure period established that the exposure solution temperatures ranged from 19 to 22°C.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

No conclusions were made by the author.

Quality Assurance and Good Laboratory Practice Regulation Statements were included in the report, indicating that the study was conducted in accordance with all pertinent EPA Good Laboratory Practice Regulations with the following exception: stability, characterization and verification of the test substance identity and maintenance of records on the test substance are the responsibility of the test sponsor.

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS

A. Test Procedure: The test procedures were generally in accordance with the SEP except for the following:

First instar Daphnia magna used in tests should be from the fourth or later broods of a given parent. The author did not specify which brood was the source of the test animals.

It was not stated whether the nonlabeled test material (41.2% purity) was a formulated product or a technical grade material. If a formulated product is tested, a formulated blank control should be included in the test design.

The report did not describe the size and construction material of the test vessels.

The report did not specify whether the photoperiod contained 15- to 30-minute transition periods between light and dark.

The test organisms were impartially distributed to the test chambers; random assignment to the test vessels is required.

The test temperature ranged from 19-22°C. The temperature should not vary more than 1°C during the entire study period.

- B. Statistical Analysis: The reviewer used EPA's Toxanal computer program to calculate the  $EC_{50}$  value and obtained the same results as the author (see attached printout).
- C. Discussion/Results: This study is scientifically sound and meets the requirements for a static acute toxicity study using freshwater invertebrates. Based on mean measured concentrations, the 48-hour  $EC_{50}$  was 94  $\mu\text{g}$  a.i./l, which classifies DDAC as very highly toxic to Daphnia magna. The NOEC was 74  $\mu\text{g}$  a.i./l mean measured concentration.
- D. Adequacy of the Study:
- (1) Classification: Core.
  - (2) Rationale: N/A.
  - (3) Repairability: N/A.
15. COMPLETION OF ONE-LINER: Yes, April 16, 1992.

DDAC  
(Daphnia magna)

NOTE: THERE WAS CONTROL MORTALITY, BUT AT LEAST ONE  
OF THE LOWER CONCENTRATIONS HAD ZERO MORTALITY.  
THEREFORE, ABBOTT'S CORRECTION IS NOT APPLICABLE.

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CONC.      NUMBER      NUMBER      PERCENT      BINOMIAL
           EXPOSED     DEAD        DEAD        PROB. (PERCENT)
120        20          20          100          9.536742E-05
74         20          0           0           9.536742E-05
51         20          0           0           9.536742E-05
31         20          1           5           2.002716E-03
19         20          0           0           9.536742E-05
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THE BINOMIAL TEST SHOWS THAT 74 AND 120 CAN BE  
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT  
CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL  
ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 94.23373

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE  
PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE  
NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

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Table 2. Measured concentrations of DDAC during the 48-hour static exposure of daphnids (*Daphnia magna*).

		Measured Concentrations ( $\mu\text{g A.I./L}$ )				Mean Measured Concentration <sup>c</sup> (SD) <sup>d</sup> ( $\mu\text{g A.I./L}$ )	% Nominal
Nominal Concentrations ( $\mu\text{g A.I./L}$ )		0-Hour <sup>a</sup>	24-Hour <sup>b</sup>	48-Hour <sup>a</sup>			
100	1	120	140	120	120(12)	-	120
	2	100	120	130	-		
	3	110	120	130	-		
60	1	71	66	92	74(9.7)	-	123
	2	80	78	79	-		
	3	66	59	72	-		
37	1	48	63	65	51(9.6)	-	138
	2	41	58	46	-		
	3	40	57	44	-		
22	1	25	39	41	31(7.1)	-	141
	2	23	36	32	-		
	3	21	35	29	-		
13	1	20	13	21	19(2.9)	-	146
	2	20	20	21	-		
	3	18	22	17	-		
Control	1	< 1.43	< 1.42	< 1.42			
	2	< 1.43	< 1.43	< 1.42			
	3	< 1.43	< 1.43	< 1.42			
QC # 1 <sup>e</sup>		96.7(92.1) <sup>f</sup>	12.6(90.0)	98.0(93.3)			
QC # 2		7.06(101)	31.6(90.3)	19.1(91.0)			
QC # 3		44.5(90.8)	98.3(93.6)	39.9(95.0)			

<sup>a</sup> Water samples used are from replicate A.

<sup>b</sup> Water samples used are from replicate B.

<sup>c</sup> Mean measured concentrations were calculated using actual analytical results (three significant figures) rather than the rounded values (two significant figures) presented in this table.

<sup>d</sup> SD = Standard Deviation.

<sup>e</sup> QC = Quality Control Sample.

<sup>f</sup> Quality Control sample results are presented with the percent recovery in parentheses.

Springborn Laboratories, Inc.

Table 3. Concentrations tested, corresponding cumulative number of immobilized organisms and observations made during the 48-hour static exposure of daphnids (*Daphnia magna*) to DDAC.

Mean Measured Concentration ( $\mu\text{g A.I./L}$ )	Cumulative Number of Immobilized Organisms %					
	24-hour			48-hour		
	A	B	Mean	A	B	Mean
120	0	0	0 <sup>a</sup>	100	100	100
74	0	0	0 <sup>b</sup>	0	0	0 <sup>ab</sup>
51	0	0	0	0	0	0
31	0	10	5	0	10	5
19	0	0	0	0	0	0
Control	10	0	5	10	0	5

<sup>a</sup> One of the surviving daphnids was observed to be lethargic.

<sup>b</sup> Two of the surviving daphnids were caught on particulate matter.